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Applicant : van der Laan, J.C. et al. Art Unit: 1814
Serial No.: 07/565,673 Examiner: Hendricks, K.
Filed : August 10, 1990
Title : EFFICIENT PRODUCTION OF MUTANT PROTEASES

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Commissioner of Patents and Trademarks
Washington, DC 20231

APPELLANTS' BRIEF ON APPEAL
SUBMITTED PURSUANT TO 37 CFR 1.192

Sir:

This is an appeal of the Examiner's Final Rejection, mailed March 2, 1993. Submitted herewith are three copies of Applicants' brief on appeal, together with the requisite fee of $270.00. Please apply any charges not covered, or any credits, to Deposit Account No. 06-1050.

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Date of Deposit March 2, 1994
I hereby certify under 37 CFR 1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated above and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Arthur L. Lapen

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(1) Status of Claims

On September 2, 1993, appellant appealed from the final rejection of claims 4-7, 9-17, 19, and 23-26, all other claims having been cancelled. After denial of appellant's proposed amendment in an Advisory Action dated September 27, 1993, claims 4-7, 9-17, 19, and 23-26 remain pending on appeal. A copy of the claims on appeal is attached as Appendix A.

(2) Status of Amendments

Appellant filed an amendment on September 2, 1993 in response to the Examiner's final rejection of appellant's claims. In an advisory action dated September 27, 1993, the Examiner indicated that the appellant's amendment would not be entered.

(3) Summary of the Invention

The claimed invention is directed to methods for preparation of alkalophilic *Bacillus* strains which do not produce detectable amounts of extracellular high alkaline protease and the methods of using these *Bacillus* strains for producing mutant high alkaline protease substantially free of indigenous high alkaline protease. Also claimed are the *Bacillus* strains produced, a detergent composition comprising as an active ingredient one or more high alkaline proteases and a method for processing laundry.

(4) Issues

The Examiner stated two grounds of rejection. (1) Each of the claims was rejected under 35 U.S.C. § 112, ¶ 1 on the grounds that the specification enabled only claims limited to methods of producing an alkalophilic asporogenic *Bacillus novo* species PB92 of minimal indigenous extracellular protease level, transformed with a mutated *B. novo* PB92 alkaline protease, and not the generic methods and compositions claimed. (2) Each of the claims was rejected under 35 U.S.C. § 103 as obvious over Fahnestock et al. and Estell et al., in view of TeNijenhuis and Suggs et al.

Appellant respectfully submits that each of these grounds of rejection was erroneous.

(5) Grouping of Claims

The rejected claims do not stand or fall together; reasons as to why appellant considers the rejected claims to be separately patentable are presented in paragraph (6) below.

(6) Argument

A. Section 112 First Paragraph Rejection

The Examiner rejected all pending claims for lack of enablement. The Examiner took the position that the specification is enabling only for claims limited to methods of producing an alkalophilic asporogenic *Bacillus novo* species PB92 of minimal indigenous extracellular protease level, transformed with a mutated *B. novo* PB92 alkaline protease. (Paper 15, at 2:1-22). This limited reading of Applicants' invention is unwarranted.

It is well-established that to be enabling, the specification need only provide sufficient information to allow one skilled in the art to make and use the invention without undue experimentation. Scripps Clinic & Research Found. v. Genentech, Inc., 927 F.2d 1565, 18 U.S.P.Q.2d 1001, 1006 (Fed. Cir. 1991). "That some experimentation may be required is not fatal; the issue is whether the amount of experimentation required is 'undue.'" In re Vaeck, 947 F.2d 488, 495, 20 U.S.P.Q.2d 1438, 1444 (Fed. Cir. 1991) (emphasis in original). Further, the specification need not teach what is well known in the art. In re Wands 858 F.2d 731, 735, 8 U.S.P.Q.2d 1400, 1402 (Fed. Cir. 1988).

In this case, the Examiner has ignored the teachings in the art and in the specification that would lead one skilled in the art who is in possession of the specification to be able to use the claimed methods. Claim 23, for example, is directed to a method for producing a mutated high alkaline protease substantially free of indigenous extracellular high alkaline protease using an alkalophilic *Bacillus* host strain which does not produce extracellular high alkaline protease. The significance of the invention is that it overcomes significant problems which have been encountered with the use of *Bacilli* as

production hosts for recombinant proteins. *Bacilli* produce and secrete a variety of proteases which tend to degrade the heterologous proteins produced. Approximately 90% of this proteolytic activity can be attributed to neutral metal protease (NPR) and serine protease (APR). While several approaches have been used to overcome this latter problem, the resulting organisms were not completely devoid of extracellular proteolytic activity. Additionally, it was thought that the use of asporogenous mutants for the production of recombinant proteases would be unsatisfactory as the introduction of a sporulation deficiency in a strain would be expected to result in decreased protease production. (See, specification p. 1, ln. 31 - p. 2, ln. 19.) With the claimed invention, Appellants have solved these problems so that not only may a mutated high alkaline protease be produced which is substantially free of indigenous extracellular high alkaline protease, the mutated enzyme is surprisingly produced in large quantities, even when the host is an asporogenous *Bacillus*. Thus it is not production of a mutant enzyme per se that is significant, but that it can be produced in the absence of indigenous protease. How to accomplish this is set forth in the specification in general terms on page 7, line 12, through page 10, line 10. The technique is exemplified in detail in the Experimental section beginning on page 15, line 26. The methodology once worked out as described by applicants is applicable to *Bacilli* other than those used in the examples. Indeed, the specification is quite clear that this process is not limited to use with a PB92 *Bacillus* host strain, which the specification describes as "an example" of the invention. (Specification, p. 12, ln. 8). Asporogenous mutant *Bacillus* strains are a further example of a group of *Bacilli* that may be used, of which the group "derived from PBT110 and its derivatives" is preferred. (Id., p. 12, ln. 29-33). Other examples are suggested in the specification. (Id., p. 12, ln. 10-21). The Examiner is wrong as a factual matter in stating that the specification is limited to one particular example. The specification in fact gives several examples, and clearly

identifies them as merely a preferred embodiment of an invention that is effective for an entire class of *Bacillus* strain hosts. The Examiner has provided no substantiation for his assertion that the claimed methods could not be used with *Bacilli* other than those exemplified. Once the technical problem of making the host cell which does not produce extracellular high alkaline protease was solved, the application of the solution to other host organisms is well within the ordinary skill of the art. Transforming the host *Bacillus* with the constructs encoding for the gene for the protease to be produced and growing the cells to produce the protease, are within the skill in the art (see the references of record such as Fahnestock et al) as are methods for producing mutated genes (see for example Gorman et al, also of record). What was not within the skill in the art prior to filing of the subject application, was preparation of the host cell in which the entire indigenous protease gene had been deleted so as to produce an organism which did not produce extracellular high alkaline protease.

The Examiner is also wrong as a legal matter in attempting to limit Applicants' claims to the preferred embodiment disclosed in the specification. It was settled long ago that:

> To demand that [Applicant] shall limit his claims . . . to materials which meet the guidelines specified for "preferred" materials in a process . . . would not serve the constitutional purpose of promoting progress in the useful arts.

In re Goffe, 542 F.2d 564, 567, 191 U.S.P.Q. 429, 431 (C.C.P.A. 1976). The Federal Circuit has reaffirmed that principle in In re Wands, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988). In that case, the Court held that the PTO had erred in rejecting an applicant's claim to methods of using a generic class of antibodies. The applicant in Wands had deposited only a cell line secreting one specific antibody. Nonetheless, the court held that the applicant was entitled to a patent covering the use of the generic class because one skilled in the art could produce other antibodies falling within the generic class for use in the method. Id. at 740, 8 U.S.P.Q. 2d at 1406-07. The same is true

here. The Examiner has provided no support for his assertion that the claimed invention would not be operative with alkalophilic *Bacillus* strains other than PB92, nor why it would not be apparent to one skilled in the art how to make and use the claimed invention, nor why undue experimentation would be required to practice the claimed invention.

Simply because not all genes encoding extracellular high alkaline proteases are known or cloned should not mean that Appellants should be limited to what they have exemplified. If that were true, others could with impunity use Appellant's inventive concept simply by substituting another protease gene as the target gene for removal, substituting another protease gene to be expressed, and substituting a *Bacillus* other than PB92 as the expression host. Appellant has shown that their invention is operable as claimed and should be entitled to their generic claims.

The specification discloses that the process of claim 23 is effective for <u>any</u> mutant proteases. (<u>Id</u>., p. 12, ln. 22 - p. 14, ln. 3). Indeed, the specification discloses that the claimed method is not even limited to expression of a mutant high alkaline protease, but that any polypeptide of interest may be expressed in the absence of indigenous extracellular high alkaline protease. (<u>Id</u>., p. 12, ln. 34 - p. 13, ln. 3). The specification describes the mutant proteases which can be expressed: those in which at least one amino acid is different from the wild type protease. (<u>Id</u>., at p. 13, ln. 3-16).

The Examiner's objection that "one of ordinary skill in the art would not be able to determine what type of mutation, how many, at what amino acid, etc., including all variations possible . . . [and] could not prophetically predict the outcome of any mutation upon the gene" (Paper 15, p. 2, ln. 16-22) misses the point of Applicants' invention. Knowing the type of mutation induced in a particular protease gene or the effect of a mutation on protease function is not required in order to practice the instant invention. The point of the invention is that <u>any</u> mutant protease can be produced more readily in a *Bacillus* strain <u>with</u>

an indigenous protease level that is reduced by deletion of the indigenous protease gene.

The limitation in claim 17 that the mutant protease differ in at least one amino acid from the indigenous protease simply restates the requirement that the protease be mutant. Methods for producing a mutant protease differing in at least one amino acid are well known in the art. Several such methods are disclosed in the specification as examples. (Specification, pp. 15-17, 19-27). The specification teaches how to achieve mutation, as the Examiner appears to concede;[1] that is all that is required for the invention. Enumerating the particular mutant strains which could be made is beyond the scope of the invention. Thus, Applicants have disclosed all that is required to use the invention, and have complied with the requirements of section 112, ¶ 1.

Independently, even were the Examiner's rejection well taken, the Examiner erred by applying that rejection to all claims. Indeed, by the terms of the enablement rejection itself, the Examiner concluded only that the application was entitled to a limited scope of claims, not that it was entirely invalid. In particular, the Examiner concluded that the specification is enabling only for claims limited to methods of producing an alkalophilic asporogenic *Bacillus novo* species PB92 of minimal indigenous extracellular protease level, transformed with a mutated *B. novo* PB92 alkaline protease. (Paper 15, at 2:1-22).

This ground does not justify rejection of claims 4, 9, 13, 15, or 16. Each of those claims is directed to a *Bacillus* strain limited to PB92 or its derivatives as the expression host, or to a method of producing such a strain. The Examiner has offered no reason for grouping these claims with the remaining claims, and the Examiner's rejection by its own terms does not apply to these claims. Certainly it would be within the skill of the art to

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[1] The Examiner does not argue that one skilled in the art would not know when she had created a mutant protease. Such an argument would have to ignore all the progress made in biotechnology in the last 15 years.

mutate other protease genes of interest and to insert them into the altered PB92 strain or its derivatives in order to obtain production of the protease of interest in the absence of indigenous extracellular high alkaline protease.

B. The Obviousness Rejection

The Examiner rejected all pending claims under 35 U.S.C. § 103 as obvious over Fahnestock et al. and Estell et al., in view of TeNijenhuis and Suggs et al. This rejection is inappropriate.

Several classes of claims are encompassed in this rejection. Claims 23, 4-7, 9-11 and 26 are directed to methods for production of a mutant high alkaline protease; claims 12-13 are directed to a method of obtaining an alkalophilic *Bacillus* strain having a reduced extracellular alkaline protease level; claims 14-16 are directed to an alkalophilic *Bacillus* strain producing a mutant high alkaline proteases; claim 17 is directed to mutant high alkaline proteases; claim 19 is directed to a detergent composition comprising as an active ingredient a high alkaline protease; claim 24 is directed to a method of preparing a detergent composition comprising a high alkaline protease as an active ingredient and claim 25 is directed to a method of processing laundry with the claimed detergent composition.

The Examiner has the burden of establishing a prima facie case of obviousness before the Board. In re Fritch, 972 F.2d 1260, 1265; 23 U.S.P.Q. 2d 1780, 1783 (Fed. Cir. 1992). In evaluating a section 103 rejection, the Board must compare the claims and the prior art in view of the inventive concept of the invention, and without the benefit of hindsight afforded by the instant application. In re Gorman, 933 F.2d 982, 986, 18 U.S.P.Q.2d 1885, 1888 (Fed. Cir. 1991). As noted above, the inventive concept is the preparation and use of alkalophilic *Bacilli* that have minimal or no indigenous extracellular alkaline protease expression for producing a mutant alkaline protease.

Fahnestock disclose use of *Bacillus* strains having reduced levels of indigenous protease for expression and secretion of heterologous polypeptides or proteins. However, unlike the

instant invention, Fahnestock inactivates the indigenous protease by adding an inactivating CAT gene sequence to the DNA of the host strain. The resulting *Bacilli* still produce extracellular protease, both serine and neutral proteases as shown in Table 2 in column 9, lines 55 through 68. This is in contrast to *Bacilli* used by Appellants, which do not produce detectable amounts of protease. See for example, page 18, lines 6-22 of the specification. As discussed on page 6, beginning at line 30 of the specification, the use of protease negative *Bacilli* offers an advantage that the protein produced will not be degraded by indigenous extracellular proteolytic activity. Several other advantages of the subject invention are also set forth, including the advantage that when alkalophilic *Bacilli* are used as expression hosts, the risk of contamination with other microorganisms is minimized due to the alkaline pH of the growth medium during fermentation, a condition which is not well tolerated by other organisms. Fahnestock do not teach or suggest the use of *Bacilli* in which the entire indigenous protease gene has been removed. Finally, nothing in Fahnestock teaches or suggests the use of an alkalophilic *Bacillus* as an expression host.

The further addition of Estell does not cure the deficiencies of Fahnestock at al. Estell discloses preparation of a *B. subtilis* strain BG84 (column 20, beginning at line 27), using N-methyl-N'-nitro-N-nitrosoguanidine (NTA) mutagenesis of *B. subtilis* I168. BG84 is sporulation deficient. In order to be a reference under section 103, the reference must be enabling for that which it is cited. Estell made a single organism that was protease deficient using chemical mutagenesis, an entirely random process. It was simply fortuitous that the desired mutant organism was obtained. It does not teach one skilled in the art how to obtain alkalophilic *Bacilli* in which the gene for extracellular high alkaline protease was deleted. In fact, Estell's own attempts to delete the neutral protease gene resulted in only one strain which lacked protease activity, but

this strain had deletions within the protease genes; the entire genes were not deleted.

The examiner is using improper hindsight reconstruction in view of Applicants' own specification. Such hindsight analysis is both unwarranted and improper. Nothing in Estell suggests any reason to delete the rest of the coding region or any way of so doing. It is only in Applicants' own specification that the means for producing *Bacilli* which are useful in the claimed method is disclosed. Furthermore, such *Bacilli* have an unexpected property: deletion of the indigenous protease coding region actually enhances production of mutant alkaline protease. (See Table 1, page 29, lines 20-44 of the specification.) Such unexpected results are evidence that Applicants' invention would not have been obvious to those skilled in the art. In re Corkill, 771 F.2d 1496, 1500, 226 U.S.P.Q. 1005, 1008 (Fed. Cir. 1985). There is nothing in Estell which suggests that use of *Bacilli* lacking the entire indigenous extracellular protease gene would produce higher levels of mutant protease. It is only through hindsight, in view of Applicants' advances beyond Estell's teachings, that the Examiner could conclude that the references teach that which is claimed. Such hindsight is improper. In re Gorman, 933 F.2d 982, 18 U.S.P.Q.2d 1885, 1888 (Fed. Cir. 1991).

Finally, as with Fahnestock, nothing in Estell et al. teaches or suggests the use of alkalophilic *Bacillus* as an expression host. In fact, Estell teaches away from the use of an alkalophilic *Bacillus* and in particular the use of asporogenic *Bacillus* strains as expression hosts. Indeed, Estell et al. conclude that "asporogenous [*Bacilli*] . . . are unsatisfactory for the recombinant production of heterologous proteins because asporogenous mutants tend to lyse during earlier stages of their growth cycle." Given this conclusion, there is no reason to believe it would be obvious to use asporogenic *Bacillus* strains as hosts for mutant alkaline proteases. Once again, the unexpected results demonstrated by Applicants -- that asporogenic *Bacilli* are particularly suitable hosts for mutant alkaline

protease -- constitute evidence that Applicants' invention would not have been obvious to those skilled in the art. See In re Corkill, 771 F.2d 1496, 1500, 226 U.S.P.Q. 1005, 1008 (Fed. Cir. 1985).

The Suggs and TeNijenhuis references do not cure the deficiencies of the primary references. Suggs teaches general methods for performing mutagenesis. TeNijenhuis disclose the presence of indigenous alkaline protease in *Bacillus novo* species PB92. Both of these things were well known in the art; neither would assist one skilled in the art in arriving at the instant invention. Neither Suggs nor TeNijenhuis fill in the critical pieces of Applicants' invention mission from the primary references, namely the removal of the entire indigenous protease gene and the use of asporogenic and alkalophilic *Bacillus* host strains.

Furthermore, the Examiner has pointed to nothing in any of the prior art references cited which would lead one skilled in the art to combine any of these references with each other. In the absence of such a suggestion in the art itself or other proof of motivation to combine the references, the Examiner's combination of these references is improper. ACS Hospital Systems, Inc. v. Montifiore Hospital, 732 F.2d 1572, 1577, 221 U.S.P.Q. 929, 933 (Fed. Cir. 1984).

Finally, even were the Examiner correct in his section 103 rejection, he has incorrectly applied it to all the claims in this invention. The section 103 rejection is directed to that part of Applicants' invention which teaches a method for production of an alkalophilic *Bacillus* strain containing no detectable extracellular high alkaline protease - namely, claims 12 and 13. The Examiner does not explain why any of the remaining claims of the invention would be rendered obvious by his analysis. In particular, claim 19 is directed to a detergent composition comprising as an active ingredient a high alkaline protease. Claim 24 is directed to a method of preparing a detergent composition comprising a high alkaline protease as an active ingredient. Claim 25 is directed to a method of

processing laundry with the claimed detergent composition. The Examiner does not even suggest anything in the art which might render these claims obvious, and therefore cannot be said to have met his burden with respect to these claims.

Further, the Examiner has improperly grouped the claims to a method for production of an alkalophilic *Bacillus* strain with claims 23, 4-11, and 26, which claim methods for production of a mutant alkaline protease; and with claim 17, which claims the mutant alkaline protease itself. Each of these claims contains elements which the Examiner has not even attempted to relate to anything found in Fahnestock, Estell, TeNijenhuis, or Suggs, and clearly none of those references teach compositions or methods for production of mutant alkaline proteases, compositions or methods for production of detergents, or methods of processing laundry. Without some attempt to relate these claims to the prior art, the Examiner cannot possibly meet his burden before the board.

In short, the Examiner has offered no reason to combine any of the references cited, and in any event has not cited any references which (alone or in combination) suggest the central inventive concept of Applicants' claimed invention.

(7) Conclusion

For the foregoing reasons, the Examiner's rejection of claims 4-7, 9-17, 19, and 23-26 was erroneous, and should be reversed.

Respectfully submitted,

Date: March 2, 1994

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Attachment: APPENDIX A - Claims as Pending

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APPENDIX A- Claims as Pending

USSN 07/565,573

4. The method according to Claim 23, wherein said *Bacillus* strain is *Bacillus novo* species PB92 or a derivative thereof.

5. The method according to Claim 23, wherein said *Bacillus* strain is an asporogenic alkalophilic *Bacillus* strain.

6. The method according to Claim 23, wherein the gene encoding said indigenous protease has been deleted by homologous or illegitimate recombination.

7. The method according to Claim 23, wherein a plasmid comprises said expression cassette.

9. The method according to Claim 7, wherein said mutant high alkaline protease is obtained from *Bacillus novo* species PB92.

10. The method according to Claim 23, wherein at least one copy of said expression cassette is integrated into the genome of said host.

11. The method according to Claim 10, wherein said host further contains at least one copy of a plasmid comprising said expression cassette.

12. A method of obtaining an alkalophilic *Bacillus* strain having no detectable extracellular high alkaline protease, said method comprising:

transforming an alkalophilic *Bacillus* strain with a cloning vector comprising the 5′ and the 3′ flanking regions but not the coding region of a gene coding for the high alkaline protease and wherein a sufficient amount of said flanking regions is present to provide for homologous recombination with an indigenous gene coding for the high alkaline protease whereby transformants are obtained;

growing said transformants under conditions whereby the replication function encoded by said vector is inactivated; and

isolating transformants identified as having no detectable extracellular high alkaline protease.

13. The method according to Claim 12, wherein said alkalophilic *Bacillus* strain is *Bacillus novo* species PB92 or a derivative thereof.

14. An alkalophilic *Bacillus* strain producing a mutant high alkaline protease substantially free of expression product of an indigenous extracellular alkaline protease gene, wherein said strain has been obtained by transforming an alkalophilic *Bacillus* strain having no detectable indigenous extracellular high alkaline protease obtained by the method according to Claim 12 or 13 with a plasmid expression vector comprising the mutant high alkaline protease gene.

15. The *Bacillus* strain according to Claim 14, wherein aid mutant alkalophilic *Bacillus* strain is a mutant of *Bacillus novo* species PB92 or a derivative thereof.

16. The *Bacillus* strain according to Claim 15, wherein said indigenous gene has been deleted by homologous or illegitimate recombination.

17. A mutant high alkaline protease produced according to the method of Claim 23 and characterized as (1) substantially free from contamination with an indigenous extracellular high alkaline protease, and (2) differing in at least one amino acid from the indigenous high alkaline protease.

19. A detergent composition comprising as an active ingredient one or more high alkaline protease prepared according to the method of Claim 23.

23. A method for production of a mutated high alkaline protease substantially free of indigenous extracellular high alkaline protease, said method comprising:

growing an alkalophilic *Bacillus* strain host having no detectable indigenous extracellular protease as a result of deletion of the gene for indigenous extracellular protease transformed with an expression cassette providing for expression of a said mutant high alkaline protease in said host, whereby said mutant high alkaline protease is produced.